

IN VITRO PROPAGATION OF PEPEROMIA AND BEGONIA SPECIES

by

SRINIVASA RAMACHANDRA

B.Sc.(Agri.), University of Agricultural Sciences

Bangalore, India, 1982

A MASTER'S THESIS

submitted in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Approved by :



Major Professor

LD
2668
.T4
1986
R35

Al1202 971282

C. 2

TABLE OF CONTENTS

	<u>Page</u>
List of Tables	iii
List of Figures	iv
Introduction	1
Literature Review	3
Literature Cited	15
Manuscript-1 : In vitro propagation of <u>P. argyreia</u> 'Watermelon' and <u>P. caperata</u> 'Emerald Ripple'	20
Manuscript-2 : In vitro propagation of <u>B. erythrophylla</u> 'Beefsteak'	38
Appendix I : Media formulations	54
Appendix II : Experimental design	56
Appendix III a : Results of experiments repeated ...	57
Appendix III b : Results of experiments repeated ...	58
Acknowledgement	59

LIST OF TABLES

		<u>Page</u>
<u>Manuscript-1:</u>		
Table-1.	Response of petiole segments of <u>P. argyreia</u> 'Watermelon' to various combinations of NAA and Kinetin in MS media after 4 weeks in culture	32
Table-2.	Response of petiole segments of <u>P. argyreia</u> 'Watermelon' 4 weeks after transfer to MSSM medium (8 weeks in culture)	33
Table-3.	Response of petiole segments of <u>P. caperata</u> 'Emerald Ripple' to various combinations of NAA and Kinetin in MS media after 4 weeks in culture	34
Table-4.	Shoot regeneration from petiole segments of <u>P. caperata</u> 'Emerald Ripple' 4 weeks after transfer to MSSM medium (8 weeks in culture)	35
<u>Manuscript-2:</u>		
Table-1.	Response of petiole segments of <u>Begonia erythrophylla</u> 'Beefsteak' to various combinations of NAA and Kinetin in MS media after 4 weeks in culture	49
Table-2.	Shoot regeneration from petiole segments of <u>B. erythrophylla</u> 'Beefsteak' 4 weeks after transfer to MSSM medium (8 weeks in culture)	50

LIST OF FIGURES

	<u>Page</u>
<u>Manuscript-2:</u>	
Figure-1. Response of <u>Begonia erythrophylla</u> 'Beefsteak' to in vitro culture	47

INTRODUCTION

The tissue culture method is becoming increasingly significant in rapid clonal propagation of tropical foliage plants (25). A million-fold enhancement per year over the more conventional methods of vegetative propagation has been reported for the multiplication of gerbera daisy using tissue culture techniques (26). The most significant advantage offered by aseptic methods of in vitro propagation (also referred to as 'clonal' or 'micro' propagation) over the conventional methods is that in a relatively short time and space a large number of plants can be produced using a single plant part.

In the past decade, the commercial benefits of tissue culture have been realised by many nurseries and numerous commercial laboratories have been set up throughout the United States and other parts of the world to carry out rapid clonal propagation of economically important plant species. The tissue culture system not only facilitates the build up of initial propagation stock (16) but also provides increased rates of propagation and can facilitate asexual multiplication of plants that had previously proved difficult or impossible to propagate. It also aids in the production of virus-free plants (23) by employing meristem-tip culture and the storage of valuable germplasm through cryopreservation techniques (1).

Tropical foliage plants have attained a very significant status economically. They are desired for use indoors as potted plants and in terraria. In recent years there has been an increase in use of foliage plants in interior design, in houses and apartments, office buildings, and shopping centers (32). These expanding demands have added tremendously to the traditional demand for foliage plants. But, commercial production has not kept pace with demand. To meet this demand, the tissue culture method of rapid vegetative propagation can be expected to substantially improve the rate of multiplication of foliage plants. Today, many foliage plant species, some flowering plants and woody ornamentals are being successfully propagated by using tissue culture techniques.

In the present study, attempts have been made to propagate two species of Peperomia and another species of Begonia using in vitro propagation techniques. Both Peperomia and Begonia are widely used as indoor ornamental plants.

LITERATURE REVIEW*

The genus Peperomia, of the pepper family Piperaceae, widely distributed in the warm parts of the world, is represented in the native flora of the United States in southern Florida. It comprises of close to 1000 species of chiefly low evergreen perennials and some annuals (9). Peperomias are best known as beautiful small foliage plants for greenhouses and terrariums, with some of the more succulents, best able to flourish in or at least tolerate dry atmospheric conditions, as indoor plants (9). They are very well suited for planting in pots, or trailing sorts in hanging baskets , and in ground beds. In the tropics and warm subtropics selected cultivars form excellent ground cover and rock garden ornamentals.

Peperomia argyreia (syn. P. andersii Hort.), popularly known as watermelon peperomia, is best described as having dark green, long-stalked, shield-shaped, hairless leaves with stalks joined to the blades well in from their margins (4,9). Nearly

* The following abbreviations have been used : AdS (Adenine disulphate); BA or BAP (6-Benzylaminopurine); 2,4-D (2,4-dichlorophenoxyacetic acid); GA (Gibberellic Acid); 2iP (2-isopentenyl aminopurine); IAA (Indole-3-Acetic Acid); IBA (Indole butyric acid); Kinetin (6-furfuryl aminopurine); MS (Murashige & Skoog); NAA (Naphthalene acetic acid); SH (Schenck & Hildebrandt).

stemless, shapely, and full-foliaged, this species, 6 to 9 inches tall, has leaf blades 2 to 3 inches long. The leaves of this Brazilian species have red stalks and fleshy blades with conspicuous lengthwise bands of green and silver reminescent of some watermelons (9).

Peperomia caperata 'Emerald Ripple', another Brazilian species, appears to be most typical of the species and is approx. 4 inches tall by 5 inches across. It has a short, branched stem and, displayed in spiraled, more or less rosette fashion, strongly rippled or corrugated leaves with pink stalks upto 3 1/2 inches long and broad-heart-shaped to ovate blades upto 1 1/2 inches across (9).

Peperomias are normally propagated by stem, leaf-bud or leaf cuttings (12). Some species of Peperomia are found to regenerate easily in vitro while others do not. Kukulczanka et al. (21) reported that leaf fragments of Peperomia scandens Ruiz. regenerated entire plantlets when cultured on a basic Murashige & Skoog (MS) medium supplemented with NAA and Kinetin. A combination of Kinetin and NAA in a ratio of 4:2 ppm resulted in the highest regeneration and shoots and roots developed on the leaf sections independent of each other. They concluded that summer and spring were the most advantageous seasons for taking the explants.

Klimazewska (19,20) carried out a study on the regenerative potential of two species of Peperomia in tissue culture and the

biological activity of endogenous growth regulators. Sections of petioles and leaf blades were cultured on MS medium supplemented with various concentrations of kinetin and/or BA in combination with NAA. All explants of P. scandens regenerated plantlets after 4 weeks in culture on the medium containing 2 ppm NAA and 2 ppm BA, whereas only callus and root formation was seen in P. eburnea even after 8 weeks in culture. The biotests revealed that the level of endogenous growth regulators in both species were not enough to initiate regeneration. Similarly Scaramello (31) reported successful propagation of P. longifolia plants in vitro.

Berry (2) reported successful regeneration of P. caperata from friable callus obtained by culture of sections from young and old leaves. The explants were placed on 81 different media combinations. The variables included 3 concentrations (designated low, medium and high) of auxins, cytokinins, vitamins and growth factors (myo-inositol, AdS, and monobasic sodium phosphate) in both light and dark. After 8 weeks of culture regeneration occurred only on 2 treatments : low or medium concentrations of vitamins plus medium concentration of growth factors, and low concentrations of both auxins and cytokinins. After 12 weeks, regeneration occurred in 13 treatments : with medium concentration of vitamins, medium concentration of growth factors, low concentration of auxins, and low or high concentrations of cytokinins showing most significant results.

Hui and Zee (15) reported an improved method of in vitro propagation of Peperomia viridis from leaf discs using MS medium supplemented with ginseng powder in addition to the hormones. Various combinations of Kinetin (0.5 to 8 mg/l) and NAA (0.5 to 2.0 mg/l) were tested. Up to 60% of regeneration was obtained with 2 mg/l kinetin + 1 mg/l NAA, but the yields were not consistent. The addition of 100-500 mg/l of raw ginseng powder increased the number of shoots per explant and improved the quality of the regenerated plantlets.

Leaf discs (1 cm²) of Peperomia 'Red Ripple' (resembles P. pseudorufescens C.DC.) were cultured on a SH medium by Henny (14). A factorial experiment was initiated with various combinations of kinetin and NAA. After 10 weeks of incubation, treatments with 2.5, 5.0 and 10.0 mg/l kinetin and 0.05 mg/l NAA or 10.0 mg/l kinetin and 0.5 mg/l NAA had produced numerous (>50) shoots at the periphery of the leaf sections. The high kinetin/NAA ratio consistently promoted shoot initiation whereas increasing the level of NAA led to excessive callus development, root formation and media discoloration. Root formation was induced by reducing the kinetin level to 0.01 mg/l while maintaining the NAA level at 0.05 mg/l.

BEGONIA:

The genus Begonia is composed of some thousands of horticultural hybrids and varieties, in addition to natural species. They are grown as flowering potted plants with

tremendous variability in flower size, form and texture (7). Plants vary from 2 inches (5 cms) to over 6 feet (1.8 meters) and are herbacious or semiwoody shrubs with root stocks that may be fibrous, bulbous, rhizomatous or tuberous (22). The asymmetrical leaves are petioled and alternate. Most plants are used as bedding plants, flowering pot plants, or foliage plants. They are usually divided into three groups : fibrous-rooted, rhizomatous, and tuberous forms. Only fibrous-rooted and rhizomatous forms are commonly used indoors (22).

Begonias are generally propagated by means of leaf cuttings. Certain cultivars, such as Christmas Begonia (B. x cheimanthia), are produced by using an entire leaf including petiole (13). But this propagation method does not allow for rapid rate of multiplication. Development of successful methods of propagation such as tissue culture which permits multiple cuttings from each leaf would facilitate commercial production of this species.

Begonia erythrophylla (syn. B. feastii) (B. manicata x B. hyrdocotyfolia), the beefsteak begonia, has creeping rhizomes and nearly-circular , thick leaves up to about 8 inches wide; their upper surfaces are lustrous dark green with paler veins, their undersides red with green veins. The pink flowers about 3/4 inch wide, are gracefully displayed in tall, erect, pyramidal panicles (8).

A factorial experiment consisting of four different concentrations of NAA and BA was carried out by Mikkelsen and Sink (24) in an attempt to propagate Rieger Elatior Begonia 'Schwabenland Red' in vitro. Leaf-petiole sections, 5 mm in length, were placed on MS media modified with various combinations of NAA and BA. A medium combination of 0.1 ppm NAA and 0.4 ppm BA was found to be optimum for the production of adventitious shoots. After 4 weeks of culture the leaf-petiole explants were sub-cultured on the same medium under 1 klx illumination. Two weeks later a yield of 45 shoots per petiole segment or 135 shoots per petiole was obtained. After 4 weeks of subculture these shoots were transferred to a rooting medium composed of MS medium modified with 0.1 ppm NAA, 2% sucrose and 1% agar and the shoots were incubated under cool-white fluorescent light at 4.5 klx. The rooted plants were later transferred to the greenhouse and a total survival of 49% was reported (i.e. from culture to established greenhouse plants).

Effects of physical and chemical factors on differentiation and growth of Begonia x hiemalis Fotsch, cultivar 'Schwabenland Red', in vitro were examined by Takayama and Misawa (34), and a mass-propagation scheme was established. In shaking-culture, differentiated buds grew rapidly into large aggregates of plantlets without roots. They were divided into plantlets and then transferred to MS agar medium modified with NAA and a higher concentration (10.0 mg/l) of sucrose for rooting. They estimated that by this method 10^{14} or more plantlets may be produced in one year from a 7 x 7 mm young leaf segment.

In an effort to study variation in two Begonia x hiemalis clones after in vitro propagation, Westerhof et. al. (38) vegetatively propagated one plant of 'Aphrodite Pink' and one of 'Schwabenland Red' in four ways. One group of plants was obtained by cutting propagation and the other by the bacterial elimination system, as developed by Hakaart and Vershijis (11), employing an in vitro propagation step. The third and the fourth groups were obtained by adding one and two cycles, respectively, of in vitro propagation after bacterial elimination, so that one, two and three propagation cycles could be compared. One cycle, followed by bacterial elimination, gave nearly uniform offspring. However, when this was followed by one or two cycles of in vitro propagation, the variation increased. Variation also increased when the size of the plantlets obtained in vitro decreased. They (38) concluded that an important source of variation can be avoided by eliminating the smallest in vitro plantlets.

Imelda (17) cultured petiole segments of Rieger Begonia on a MS medium with different combinations of NAA, BAP + 2iP and sometimes GA₃. Survival of the explants and organ and plantlet formation were affected by the presence of exogenous growth substances. The combination of 0.1 mg/l NAA and 0.2 or 0.4 mg/l BAP + 2iP appeared to be optimum for the production of shoots and roots whereas the subsequent omission of cytokinin and the addition of a low level of GA₃ was able to induce better quality plantlets. Various cultivars and types of explants responded differently to the exogenous hormones, but the cultivar Baluga showed a slightly better response.

The origin and morphology of organs was studied by Welander (37) in petiole explants of Begonia elatior hybr., grown on an agar medium supplemented with 0.1 mg/l NAA and 0.05 mg/l BAP. The explants were of four kinds : (a) epidermis, collenchyma, parenchyma and vascular tissues, (b) parenchyma and vascular tissues, (c) as in (a) but with only small areas of epidermis left, and (d) epidermis and collenchyma. Shoots and roots were both initiated in all types of explants that contained epidermis and collenchyma. Roots were also formed from parenchyma. The organs formed mostly at the morphological base when it was directed upwards or downwards. In explants placed "sideways" organs were more often located at both ends of the same explants.

There have been significant reports on the field performance of tissue-culture-derived plants. A large scale field experiment was set up by Samyn et al. (30) with selected Begonia x tuberhybrida 'Multiflora' cultivars to compare the development of plantlets obtained by tuber propagation method to plantlets derived from tissue culture. The yield of tubers indicated that with tissue-culture-derived plants, a commercial tuber can be produced within one growing season. Plant quality and size were significantly increased compared with those from the classic vegetative propagation method of virus-infected cuttings.

Petiole explants from 17 cultivars of Begonia x hiemalis were grown on a basal agar medium with different combinations of NAA and BA (36). The stock plants were kept either under short days (7-8 h of light per day) at 15°C or under long days (15-16 h

of light per day) at 18-21°C. The day length and the temperature during the in vitro culture was 20 h of light and 21°C respectively. Explants from short-day treated stock plants did not show any differentiation. In explants from long-day treated stock plants, the percentage of explants with shoots and roots varied with NAA and BA concentrations. Plantlet formation was frequent in plants from NAA:BA ratios of 2:1 and 10:1, and a variation was found between different cultivars.

Fonnesbech (9) studied the effect of exogenously supplied NAA and BA on the shoot and root formation in isolated petiole segments of Begonia x cheimantha in vitro on a modified White's medium. The best development of normally appearing plants was obtained on media containing 0.01 mg/l NAA and 0.5 to 1.0 mg/l of BA. Lower concentrations of BA yielded no shoots, whereas the higher concentrations promoted shoot formation, but the shoots were abnormal with malformed leaves. Temperature proved to be of utmost importance for the induction of shoot formation. Thus significantly fewer shoots were formed at higher temperature (25°C) than at lower temperatures (15 to 20°C).

An in vitro method of propagating Begonia x tuberhybrida from leaf sections was carried out by Peck & Cumming (28) in which leaf sections were partially submerged in a semisolid MS medium supplemented with 1.0 mg/l NAA and 5.0 mg/l BA. After 8 to 10 weeks, explants showing bud differentiation were divided and subcultured on MS liquid medium containing 1.0 mg/l NAA and BA to induce proliferation of propagules. The propagules were

transferred in vivo to an IBA solution for 10 days to induce rooting. The total time lapse between taking the explants and transfer of rooted plantlets was just over 5 months.

OTHER SUCCULENT ORNAMENTALS :

Kevers et al. (18) reported the rapid development of axillary buds from shoot tips and nodes of 18 cultivars of Fuschia hybrida on solid MS medium with BAP (1 mg/l) and an auxin (0.1 mg/l). NAA as the auxin appeared to be more active than IAA or IBA. Agitated and non-agitated liquid media of the same composition were less effective.

Cassells and Plunkett (5) carried out production and growth analysis of plants from leaf cuttings, and from tissue cultures of disks from mature and young axenic leaves of African Violet (Saintpaulia ionantha). Relatively high adventitious bud numbers were obtained on a MS basal medium containing 1 mg/l NAA and 1 mg/l BAP for both explant types. It was found that the growth of adventitious shoots was facilitated if leaf disks were transferred after bud formation to hormone-free medium. The growth analysis of the progeny plant populations showed differences in plant diameter, leaf number, leaf area and petiole length between tissue culture progenies and those derived from leaf cuttings. Intra-population variation was high for all three progeny populations.

The role of NAA during in vitro seed germination and subsequent growth in three Bromeliaceae was examined by

Pierik et al. (29). NAA incorporation in the medium resulted in a strong stimulation of root and shoot growth, with an optimum response at 0.5 - 0.8 mg/l. They (29) hypothesized that the usually slow growth of bromeliad seedlings in soil is primarily due to poor rooting, which is the result of auxin deficiency.

Leaf explants of Sansevieria trifasciata (snake plant) cultured in vitro on a modified MS medium containing 0.25 mg/l 2,4-D produced masses of meristemoids (3). The meristemoids formed shoots when transferred to a similar medium containing 0.3 mg/l Kinetin. Peck and Cumming (27) cultured corollas of cape primrose flowers (Streptocarpus x hybridus) on a MS medium modified with adenine, L-tyrosine, NAA and BA. They reported the production of areas of green callus in 5 weeks and bud formation from these areas within another 3 weeks. Rooted shoots were obtained within 12 to 16 weeks after initial culture.

Torres and Natarella (35) reported multiple shoot formation from terminal and lateral buds excised from stems of Exacum affine on a MS medium containing 0.01 mg/l NAA and 2.0 mg/l kinetin.

As many as one million shoots per year could, theoretically, be obtained from in vitro cultured shoots of Gardenia jansinoides as reported by Dumanois et. al. (6). Axillary shoots were produced in the presence of BA (0.3 to 1.0 mg/l) and IAA (1mg/l) and rooting occurred easily in 3 weeks in vitro in the presence of IAA (1.0 mg/l) and charcoal (2mg/l) or in vivo by soaking the base of the shoots in the same auxin for 2 hours. Stolz (33)

reported that utilizing rapidly replicating cultures for continued propagation and slow cultures for producing plants of Hydrangea macrophylla 'Merveille' was an effective method of increasing the overall tissue culture propagation rate.

LITERATURE CITED

1. Bajaj, Y.P.S. and J. Reinert. 1977. Cryobiology of plant cell cultures and establishment of gene-banks. In: J. Reinert and Y.P.S. Bajaj (eds). Plant Cell, Tissue and Organ Culture. Springer-verlag, Berlin, pp 757-776.
2. Berry, S. 1977. Regeneration of Peperomia caperata from callus. In vitro 13 (3) : 145 (abstr.).
3. Blazich, F.A. and R.T. Novitzky. In vitro propagation of Sansevieria trifasciata. HortScience 19 (1) : 122-123.
4. Boodley, J.W. 1981 (ed.) The commercial Greenhouse. Delmar Publishers, New York, 568p.
5. Cassells, A.C. and A. Plunkett. 1984. Production and growth analysis of plants from leaf cuttings, and from tissue cultues of disks from mature leaves and young axenic leaves of African Violet (Saintpaulia ionantha Wendl.). Scientia Hortic. 23 : 361-369.
6. Dumanois, C., B. Gobin and C. Bigot. 1984. In vitro vegetative propagation of Gardenia jansinoides Ellis. J. Plant Physiol. 116 (5) : 389-407.
7. Everett, T.H. (ed.). 1981. In : The New York Botanical Garden Ill. Encyclopedia of Horticulture. 2 : 366-394.
8. Everett, T.H. (ed.). 1981. In : The New York Botanical Garden Ill. Encyclopedia of Horticulture. 8 : 388.

9. Everrett, T.H. (ed.). 1981. In : The New York Botanical Garden Ill. Encyclopedia of Horticulture. 8 : 2553-2558.
10. Fonnesbech, M. 1974. The influence of NAA, BA and temperature on shoot and root development from Begonia x cheimantha petiole segments grown in vitro. *Physiol. Plant.* 32 : 49-54.
11. Hakaart, F.A. and J.M.A. Vershijis. 1983. Control of leaf curl and Xanthomonas begoniae in Begonia 'Elatior' by meristem culture and an isolation test. *Acta Hortic.* 131 : 299-301.
12. Hartman, H.T. and D.E. Kester. (eds). 1983. *Plant Propagation : Principles and Practices*. Prentice-Hall, Inc., New Jersey, pp 709.
13. Heide, O.M. 1964. Effects of light and temperature on the regeneration ability of *Begonia* leaf cuttings. *Physiol. Plant.* 17 : 789-805.
14. Henny, R.J. 1978. In vitro propagation of Peperomia 'Red Ripple' from leaf discs. *HortScience* 13 (2) : 150-151.
15. Hui, L.H. and S.Y. Zee. 1981. In vitro propagation of Peperomia viridis using medium supplemented with Ginseng powder. *HortScience* 16 (1) : 86-87.
16. Hussey, G. 1978. The application of tissue culture to the vegetative propagation of plants. *Sci.Prog.* 65 : 185-208.

17. Imelda, M. 1983. Tissue culture of Rieger Begonia petiole segments. *Annales Bogorienses* VIII (1) : 1-11.
18. Kevers, Cl., M.F. Coumans-Gilles, M. Coumans and Th. Gaspar, 1983. In vitro vegetative multiplication of Fuschia hybrida. *Scientia Hortic.* 21 : 67-71.
19. Klimazewska, K. 1979. The regenerative potential of two species of Peperomia in tissue cultures and biological activity of endogenous growth regulators. *Acta Hortic.* No. 91 : 281-286.
20. Klimazewska, K. 1981. Plant regeneration from petiole segments of some species in tissue culture. *Acta Agrobotanica* 34 (1) : 5-28.
21. Kukulczanka, K., K. Klimazewska and H. Pluta. 1977. Regeneration of entire plants of Peperomia scandens Ruiz. from different parts of leaves in vitro. *Acta Hortic.* No. 78 : 365-371.
22. McConnell, D.B., R.W. Henley, R.L. Biamonte. 1981. Commercial Foliage Plants. In : J.N. Joiner (ed.) *Foliage Plant Production*. Prentice-Hall, Inc., New Jersey, pp 552-553.
23. Mellor, F.C. and R. Stace-Smith. 1977. Virus-free potatoes by tissue culture. In : J. Reinert and Y.P.S. Bajaj (eds.) *Plant Cell, Tissue and Organ culture*. Springer-Verlag, Berlin, pp 616-636.

24. Mikkelsen, E.P. and K.C. Sink. 1978. In vitro propagation of Rieger Elatior Begonias. HortScience 13 (3) : 242-244.
25. Miller, L.R. and T. Murashige. 1976. Tissue culture propagation of tropical foliage plants. In vitro 12 : 797-813.
26. Murashige, T., M. Serpa and J.B. Jones. 1974. Clonal multiplication of Gerbera through tissue culture. HortScience 9: 175-180.
27. Peck, D.E. and B.G. Cumming. 1984. In vitro vegetative propagation of cape primrose using the corolla of the flower. HortScience 19 (3) : 399-400.
28. Peck, D.E. and B.G. Cumming. 1984. In vitro propagation of Begonia x tuberhybrida from leaf sections. HortScience 19 (3) : 395-397.
29. Pierik, R.L.M., H.H.M. Steegmans and J. Hendricks. 1984. The influence of Naphthalene acetic acid on the growth of in vitro cultivated seedlings of Bromeliaceae. Scientia Hortic. 24 : 193-199.
30. Samyn, G.L., P.C. Debergh and D. Vermaerke. 1984. Field performance and phenotypic stability of virus-free tissue-cultured Begonia x tuberhybrida 'Multiflora'. Scientia Hortic. 24 : 185-191.

31. Scaramello, P. 1978. Embryogeny in Piper nigrum and Peperomia longifolia explants. Fourth Intern. Congr. Plant Tissue Cell Culture, p43.
32. Smith, C.N. and E.T. Scarborough. 1981. Status and development of Foliage Plant Industries. In : Jasper N. Joiner (ed.) Foliage Plant Production. Prentice-Hall, Inc., New Jersey, pp 1-37.
33. Stoltz, P. 1984. In vitro propagation and growth of hydrangea. HortScience 19 (5) : 717-719.
34. Takayama, S. and M. Misawa. 1982. Factors affecting differentiation and growth in vitro and a mass-propagation scheme for Begonia x hiemalis. Scientia Hortic. 16 : 65-75.
35. Torres, K.C. and N.J. Ntarella. 1984. In vitro propagation of Exacum. HortScience 19 (2) : 224-225.
36. Welander, T. 1977. In vitro organogenesis in explants from different cultivars of Begonia x hiemalis. Physiol. Plant. 41 : 142-145.
37. Welander, T. 1981. Effect of polarity on and origin of in vitro formed organs in explants of Begonia elatior hybr. Swedish J. Agric. Res. 11 : 77-83.
38. Westerhoff, J., F.A. Hakaart and J.M.A. Vershijis. 1984. Variation in two Begonia x Hiemalis clones after in vitro propagation. Scientia Hortic. 24 : 67-74.

MANUSCRIPT-1

This manuscript is written in the style of
and for publication in the Journal of the
American Society for Horticultural Science

IN VITRO PROPAGATION OF PEPEROMIA ARGYREIA 'WATERMELON' AND
PEPEROMIA CAPERATA 'EMERALD RIPPLE'¹

S. Ramachandra and H. Khatamian ²

Department of Horticulture
Kansas State University, Manhattan, Kansas 66506

Additional index words : tissue culture, foliage plants, growth regulator requirements, peperomia

Abstract

Leaf discs and petiole sections of 'Watermelon' and 'Emerald Ripple' peperomia were cultured in vitro on MS medium supplemented with various concentrations of NAA (0.0 to 5.0 mg/l) and Kinetin (0.0 to 10.0 mg/l). After four weeks, all explants were transferred to MS shoot multiplication medium supplemented with 80 mg Adenine sulfate and 30 mg 2iP/liter. 'Watermelon' peperomia showed a poor in vitro shoot formation as compared to 'Emerald Ripple' except for the highest concentration of NAA and Kinetin. 'Emerald Ripple' showed good shoot formation in treatments containing 10.0 mg kinetin + 1.0 mg NAA followed by 5.0 mg kinetin + 2.0 mg NAA. Complete plantlets were obtained by 12 weeks and were transferred to sterile vermiculite medium in the growth chamber. Increasing NAA and Kinetin concentrations promoted shoot formation in general.

¹ Received for publication _____ Contribution No. _____
Department of Horticulture, Kansas State University, Manhattan,
KS 66506.

² Graduate student and Associate Professor

The tissue culture method of rapid vegetative propagation is widely used for the commercial production of many tropical ornamental plants (6,12). Peperomias, members of the Piperaceae family, are native to the tropical America (16). They are traditionally propagated through stem, leaf-bud, or leaf cuttings (4).

Some species of peperomia are reported to have been propagated through tissue culture. Peperomia scandens regenerated plantlets after 4 weeks in culture on MS medium containing 2ppm NAA and BA whereas only callus and root formation was seen in P. eburnea even after 8 weeks in culture (9,12). The addition of 100-500 mg/l of raw ginseng powder increased the number of shoots per explant and improved the quality of regenerated plantlets from leaf discs of P. viridis (7). Peperomia 'Red Ripple', a relatively new cultivar, produced numerous (>50) shoots at the periphery of leaf sections when cultured on Schenck & Hildebrandt (SH) medium supplemented with NAA and kinetin (5). P. longifolia is also reported to have regenerated whole plantlets in culture (15).

Attempts to regenerate P. sandersii from leaf squares treated with auxins and cytokinins have failed (3). Leaf cuttings of P. argyreia 'Watermelon' did not initiate shoots easily when treated with BA and IBA and increasing BA concentrations were found to be inhibitory to shoot development (2). Berry (1) reported successful regeneration of P. caperata from friable callus obtained by culture of sections from young

and old leaves. P. caperata 'Emerald Ripple' was selected as a second cultivar to compare its response to in vitro culture with that of P. argyreia 'Watermelon'.

The objectives of the present study were to regenerate plants from petiole and leaf sections of P. argyreia 'Watermelon' and P. caperata 'Emerald Ripple' using tissue culture methods and suggest a procedure for the tissue culture propagation of the described species.

MATERIALS AND METHODS

1. **Stock Plants** : Stock plants of P. argyreia 'Watermelon' and P. caperata 'Emerald Ripple' were maintained in a growth chamber with 16h photoperiod and a constant temperature of 24°C. They were kept well-watered and fertilized with a liquid solution of 20:20:20.

2. **Explants** : Young, fully expanded leaves (with petioles) were excised from the stock plants of both species and were washed gently with a detergent and running tap water. The surface sterilization procedures were carried out under a laminar flow hood. The washed leaves were dipped in 70% ethanol for 10 seconds and ethanol was allowed to evaporate in the sterile hood. They were then transferred to 20 % chlorox (5.25 % sodium hypochlorite) solution in 0.1 % Tween-20 for 15 minutes. The leaves were then repeatedly washed in sterile water to remove the excess chlorox. Petiole segments (5 mm length) and leaf discs (5 mm diameter) were excised from the sterile leaves and transferred to the nutrient medium.

3. **Media Formulations** : The basal medium consisted of Murashige & Skoog (MS) (13) salts supplemented with 0.5 mg Nicotinic acid, 0.5 mg Pyridoxine.HCl, 0.1 mg Thiamine.HCl, 100 mg m-inositol, 30 g sucrose and 10 g agar per liter and with various combinations of NAA (0, 0.5, 1.0, 2.0 and 5.0 mg/l) and Kinetin (0, 1.0, 2.0, 5.0 and 10.0 mg/l) concentrations. The pH of the medium was adjusted between 5.6 - 5.7 with 0.1N KOH and autoclaved at 15 psi and 121°C for 20 minutes. Twenty-five ml aliquots were

distributed into 25 x 150 mm sterile test tubes and cooled down to room temperature in a slanting position. A 5 x 5 diallele experiment was employed with 25 treatment combinations and 3 replications.

4. Culture conditions : All culture tubes were placed in an incubator with a constant temperature of 24 °C and light intensity of 1 Klx. Four weeks after initial culture all explants were transferred to a MS shoot multiplication medium (MSSM) consisting of basal MS medium supplemented with 80 mg Adenine sulfate and 30 mg 2i-p/l. Observations were recorded on the amount of callus formed and the number of shoots/roots formed. After 8 weeks of culture the shoots were transferred to a MS rooting medium consisting of basal salts supplemented with 8 g agar, 30 g sucrose and 2 mg IAA/l. Root formation was observed in 2-3 weeks.

5. Transfer of plants to soil : The whole plantlets were transferred to small plastic packs containing 1.5 x 1.5 sq. inch cells (6 cells/pack) filled with sterile vermiculite and placed in big plastic boxes in the growth chamber. The boxes were lined with wet paper towels at the bottom and were covered with a ground glass plate to maintain high humidity inside the box. The plantlets were regularly fed with a 10% Hoagland's nutrient solution (8) at every watering. The glass plate was gradually lifted up over a period of 2 weeks to expose the plantlets to the growth chamber environment which consisted of a constant temperature of 24 °C, a 16 h photoperiod and 70% relative

humidity. The hardened plantlets were later transferred to bigger plastic pots containing Jiffy-mix and transferred to the greenhouse.

RESULTS AND DISCUSSION

Preliminary trials showed that petiole sections responded well to culture both in terms of callus formation and survival in culture. Leaf discs, in general, turned brown and were dead within the first three weeks of culture. Henceforth, petiole sections were used for all experiments.

P. argyreia 'Watermelon' : During the first four weeks of culture the petiole explants showed a poor response in general (table 1). There was no response at any concentration of kinetin with 0.0 or 5.0 mg NAA/liter. The explants at the above concentrations became necrotic by 3 weeks. Visual evaluation showed that callus formation occurred at all concentrations of kinetin with 2.0 mg NAA. Callus formation was also seen at 10.0 mg kinetin + 1.0 or 2.0 mg NAA. Along with callus, root formation was observed in explants at 5.0 mg Kinetin and 0.5, 1.0 or 2.0 mg NAA. Callus and roots were also seen in explants at 2.0 mg Kinetin + 1.0 mg NAA. The treatment containing 10.0 mg kinetin + 2.0 mg NAA resulted in best callus formation. The callus was bright green and approximately three times the size of the initial explant at that concentration.

After four weeks, all the explants were transferred to MS shoot multiplication (MSSM) medium containing the basal medium

supplemented with 80 mg Adenine sulfate (AdS) and 30 mg 2iP/liter. The culture conditions with respect to temperature and photoperiod were kept the same as above. At the end of the 8th week, observations were recorded on the number of shoots regenerated (table 2). Shoot formation (approximately 14 shoots/petiole section) was seen only in the explant from the treatment containing 10.0 mg kinetin + 2.0 mg NAA. Most of the other treatments showed no response except for treatments with 1.0, 2.0 or 5.0 mg kinetin + 2.0 mg NAA which showed root formation with callus. The treatment with 2.0 mg NAA and no kinetin produced only callus with no organ formation. Excessive root formation was seen in the treatment with 5.0 mg kinetin + 2.0 mg NAA.

The shoots obtained from the treatment with 10.0 mg kinetin + 2.0 mg NAA were transferred after 8 weeks to MS rooting medium (MSRM). Root formation was seen at the end of the 11th week. After 12 weeks the rooted plantlets were transferred to plastic cells containing sterile vermiculite and acclimatized to the growth chamber environment.

The results showed that 'Watermelon' peperomia, as compared to 'Emerald Ripple' peperomia, had a decreased capacity for regeneration in vitro and that a very high concentration of kinetin was required for any kind of bud/shoot initiation. This is supported to some extent by the results of Harris and Hart (3) who reported that bud formation occurs in leaf squares of P. sandersii (syn. P. argyreia) treated with very high

concentrations of kinetin (25 mg/liter) and grown on a filter paper placed on an agar slant. But to date there have been no reports of complete plantlets being obtained in vitro from explants of P. argyreia 'Watermelon'. So it has been observed that complete plantlets can be obtained from petiole sections of 'Watermelon' peperomia only when cultured on a medium supplemented with a high concentration of kinetin (10.0 mg/liter) + 2.0 mg NAA/liter. The poor response of 'Watermelon' as compared to 'Emerald Ripple' can possibly be attributed to the fact that both of them belong to different species and are genetically different from each other. Klimazewska (9) made similar conclusions for the poor response of P. eburnea as compared to P. scandens cultured in vitro. It can also be postulated that the level of endogenous growth regulators were not high enough in 'Watermelon' as compared to 'Emerald Ripple'. This premise is supported by biotests carried out by Klimazewska (9) for P. scandens and P. eburnea that revealed that the level of endogenous growth regulators were not high enough to initiate regeneration. Further research is necessary to assess the level of endogenous growth regulators using bio-assay techniques for both 'Watermelon' and 'Emerald Ripple'. This should shed some light on the differences in response to regeneration in both the species. Consistent results were obtained when the experiments were repeated under the same culture conditions (Appendix IIIa).

P. caperata 'Emerald Ripple' : 'Emerald Ripple' on the other hand showed a positive response to culture during the initial 4

weeks in terms of callus/shoot formation (table 3). No organogenesis was observed on the control medium with no growth substances. There was no response to culture at 5.0 mg NAA with any or all concentrations of kinetin. The explants in these treatments remained green for 10 days but later turned necrotic and died. On the other hand green callus and some shoot formation was observed in the following treatments : 1.0 or 2.0 mg NAA + 2.0 mg kinetin; 0.5, 1.0 or 2.0 mg NAA + 5.0 mg kinetin and 1.0 mg NAA + 10.0 mg kinetin. The callus formed in these treatments appeared bright green and were approximately twice the size of the initial explant. The shoot-like structures appeared as green protruberances which, in some of the treatments, later developed into fully formed shoots after transfer to a shoot induction medium. In one treatment (10.0 mg kinetin + 0.0 mg NAA) shoot-like protruberances appeared directly on the petiole explants, but did not develop into fully grown shoots even after transfer. The explants in the some of the other treatments produced only callus without any morphogenic responses.

The high kinetin/NAA ratio consistently promoted callus and shoot formation whereas increasing the NAA concentration to 5.0 mg/liter led to necrosis and death of the explants. The best callus/shoot formation was observed in the treatment containing 10.0 mg kinetin + 1.0 mg NAA followed by 5.0 mg Kinetin + 2.0 mg NAA (table 3).

After four weeks the explants were transferred to MS shoot multiplication (MSSM) medium containing the basal medium

supplemented with 80 mg Adenine sulfate (AdS) and 30 mg 2iP/liter. The same culture conditions were maintained with 24°C constant temperature and 16h photoperiod. Observations were recorded again at the end of the 8th week (4 weeks after transfer). Results are shown in table-4. Fully formed shoots were obtained at all, but the highest concentration of NAA with 5.0 or 10.0 mg Kinetin. Treatments with 2.0 mg Kinetin + 1.0 or 2.0 mg NAA also produced shoots. The best shoot formation (9.67 ± 2.08 shoots/ petiole section) was observed in the treatment with 10.0 mg kinetin + 1.0 mg NAA, followed by 5.0 mg Kinetin + 2.0 mg NAA (7.33 ± 1.15 shoots/petiole section).

The results showed that 'Emerald Ripple' peperomia can be successfully propagated in vitro. These results are in agreement with those obtained with other species and cultivars of Peperomia (2,5,7,9). Henny (5) reported that a combination of 10 mg/l Kinetin and 0.5 mg/l NAA produced numerous (>50) shoots in leaf sections of Peperomia 'Red Ripple'. In the present experiment similar results were obtained with 'Emerald Ripple' peperomia where approximately 10 shoots/petiole section (>60 shoots/petiole) were obtained using 10.0 mg/l Kinetin and 1.0 mg/l NAA treatment. 'Watermelon' peperomia also gave similar results at the highest kinetin concentration (10 mg/l) with 2.0 mg/l NAA. The high kinetin/NAA ratio consistently promoted shoot formation which substantiates the results of Hui & Zee (7) for P. viridis and of Henny (5) for Peperomia 'Red Ripple'. The findings of Berry (2) for P. caperata that a combination of high concentration of cytokinins and low concentration of auxin showed most significant

results in terms of regeneration further support the results of this experiment.

The results on shoot formation for both species of Peperomia are comparable to those obtained by Klimazewska (9) for P. scandens. Excessive callus and root formation that was observed in some treatments for P. argyreia 'Watermelon' is supported by similar results obtained by Klimazewska (9) for P. eburnea which showed only callus and root formation even after 8 weeks in culture. The high Kinetin/NAA ratio in general induced shoot and callus formation. Transfer of explants to the MS shoot multiplication medium (MSSM) greatly influenced shoot formation. This can possibly be attributed to the presence of Adenine Sulfate and 2iP in the MSSM. Consistent results were obtained when the experiments were repeated under the same culture conditions for 'Emerald Ripple' (Appendix IIIb).

Table 1 : Response of petiole segments of P. argyreia
'Watermelon' to various combinations of NAA and Kinetin in MS
media after 4 weeks in culture

Kinetin concn. (mg/liter)	NAA concn. (mg/liter)				
	0.0	0.5	1.0	2.0	5.0
0.0	NR*	NR	NR	callus	NR
1.0	NR	NR	NR	callus	NR
2.0	NR	NR	c/r@	callus	NR
5.0	NR	c/r	c/r	c/r	NR
10.0	NR	NR	callus	callus	NR

* = No Response; @ = Callus/roots.

Table-2 : Response of petiole segments of P. argyrea

'Watermelon' 4 weeks after transfer to MSSM* medium (8 weeks in culture)

Kinetin concn. (mg/liter)	NAA concn. (mg/liter)				
	0.0	0.5	1.0	2.0	5.0
0.0	-- ¹	--	--	callus	--
1.0	--	--	--	c/r ²	--
2.0	--	--	NR ³	c/r	--
5.0	--	NR	NR	roots	--
10.0	--	--	NR	shoots (14 ± 2.0) ⁴	--

* = MS shoot multiplication

¹ = dead explant; ² = callus/roots; ³ = No Response

⁴ = mean shoot number/petiole segment ± standard deviation

Table-3 : Response of petiole segments of P. caperata 'Emerald Ripple' to various combinations of NAA & Kinetin in MS media after 4 weeks in culture

Kinetin concn. (mg/liter)	NAA concn. (mg/liter)				
	0.0	0.5	1.0	2.0	5.0
0.0	NR*	NR	callus	callus	NR
1.0	NR	NR	callus	callus	NR
2.0	NR	callus	c/s@	c/s	NR
5.0	callus	c/s	c/s	c/s	NR
10.0	shoots	callus	c/s	callus	NR

* = No Response; @ = callus/shoots

Table-4 : Shoot regeneration from petiole segments of
P. caperata 'Emerald Ripple' 4 weeks after transfer to MSSM*
 medium (8 weeks in culture)

Kinetin concn. (mg/liter)	Mean shoot number/petiole segment				
	NAA concn. (mg/liter)				
	0.0	0.5	1.0	2.0	5.0
0.0	-- ¹	--	--	--	--
1.0	--	--	--	--	--
2.0	--	--	2.0 \pm 1.0 ²	2.33 \pm 1.53	--
5.0	2.33 \pm 1.15	2.67 \pm 1.15	5.33 \pm 0.57	7.33 \pm 1.15	--
10.0	2.33 \pm 1.15	4.33 \pm 1.15	9.67 \pm 2.08	3.33 \pm 0.58	--

* = MS shoot multiplication

¹ = dead explant; ² = mean shoot number \pm standard deviation

LITERATURE CITED

1. Berry, S. 1977. Regeneration of Peperomia caperata from callus. In vitro 13 (3) : 145 (abstr.).
2. Chinonge, K.R. 1983. The effect of N⁶-Benzyladenine and Indole-3-butyric acid on the propagation of Peperomia argyreia 'Watermelon' and Peperomia caperata 'Emerald Ripple'. M.S. Thesis. Kansas State University, Manhattan, 41p.
3. Harris, G.P. and E.M.H. Hart. 1964. Regeneration from leaf squares of Peperomia sandersii A. D.C.: A relationship between rooting and budding. Ann. Bot., N.S.Vol.28 (111): 509-525.
4. Hartman, H.T. and D.E. Kester (eds). 1983. Plant Propagation : Principles and Practices. (Third edition).Prentice-Hall, Inc., New Jersey, pp 709.
5. Henny, R.J. 1978. In vitro propagation of Peperomia 'Red Ripple' from leaf discs. HortScience 13 (2) :150-151.
6. Holdgate, D.P. 1977. Propagation of Ornamentals by tissue culture, p.18-43. In: J. Reinert and Y.P.S. Bajaj (eds.). Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-Verlag, Berlin.
7. Hui, L.H. and S.Y. Zee. 1981. In vitro propagation of Peperomia viridis using medium supplemented with Ginseng powder. HortScience 16 (1) : 86-87.
8. Johnson, C.M., P.R. Stout, T.C. Broyer and A.B. Carlton. 1957. Comparative chlorine requirements of different plant

- species. Plant & Soil 8 : 337-353.
9. Klimazewska, K. 1979. The regenerative potential of two species of Peperomia in tissue cultures and biological activity of endogenous growth regulators. Acta Hortic. No. 91 : 281-286.
 10. Klimazewska, K. 1981. Plant regeneration from petiole segments of some species in tissue culture. Acta Agrobotonica 34 (1) : 5-28.
 11. Kukulczanka, K., K. Klimazewska, and H. Pluta. 1977. Regeneration of entire plants of Peperomia scandens Ruiz. from different parts of leaves in vitro. Acta Hortic. No.78 : 365-371.
 12. Miller, L.R. and T. Murashige. 1976. Tissue culture propagation of tropical foliage plants. In vitro 12 : 797-813.
 13. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phyiol. Plant. 15 : 473-479.
 14. Murashige, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25 : 135-166.
 15. Scaramello, P. 1978. Embryogeny in Piper nigrum and Peperomia longifolia explants. Fourth Intern. Congr. Plant Tissue Culture, p.43.
 16. Watkins, J.V. and T.J. Sheehan. 1975. Florida Landscape Plants. Univ. Press of Florida, Gainesville, p.125.

MANUSCRIPT-2

This manuscript is written in the style of
and for publication in the Journal of the
American Society for Horticultural Science

IN VITRO PROPAGATION OF BEGONIA ERYTHROPHYLLA 'BEEFSTEAK'¹

S. Ramachandra and H. Khatamian ²

Department of Horticulture
Kansas State University, Manhattan, Kansas 66506

Additional index words : tissue culture, foliage plants, growth regulator requirements, begonia

Abstract

Leaf discs and petiole segments of 'Beefsteak' begonia were cultured in vitro on MS medium supplemented with various concentrations of NAA (0.0 to 5.0 mg/l) and Kinetin (0.0 to 10.0 mg/l). After four weeks, all explants were transferred to MS shoot multiplication medium supplemented with 80 mg Adenine sulfate and 30 mg 2iP/liter. Abundant shoot formation was observed with 0.5, 1.0 or 2.0 mg NAA/l and 2.0, 5.0 or 10.0 mg Kinetin/l. More than 10 shoots/petiole segment were obtained in the treatment with 10.0 mg Kinetin and 0.5 mg NAA. Whole plantlets were successfully transferred to 10 x 10 sq. cm plastic pots filled with sterile vermiculite and were grown in a greenhouse with a 100 % survival rate.

¹ Received for publication _____ Contribution No. _____
Department of Horticulture, Kansas State University, Manhattan,
KS 66506.

² Graduate student and Associate Professor.

Tissue and organ culture are now well established as methods for the rapid multiplication of many ornamental plants (5). The tissue culture system facilitates build-up of initial propagation stock (6,11), the production of virus-free plants (9) and the storage of valuable germplasm (1). It is well known that it is possible to modify the regenerative properties of plant tissues in culture in such a way that either shoots or roots become dominant. Such a modification can be achieved by the addition of different growth substances to the medium (14).

In vitro organogenesis has been studied in various species of Begonia. Effects of physical and chemical factors on differentiation and growth of Begonia x hiemalis Fotsch cv. 'Schwabenland Red' in vitro were examined and a mass-propagation scheme was established (15). The origin and morphology of organs were studied in petiole explants of Begonia elatior hybr., grown on an agar medium supplemented with Naphthalene acetic acid (NAA) and Benzyl aminopurine (BAP) (16). The influence of temperature on shoot formation has also been studied in B. x cheimantha (3) and in B. x hiemalis (4). Many of the adventitious buds of Rieger Elatior Begonias formed in vitro remain too small to be rooted, and considerable plant loss occurs at the rooting and acclimatization stages (10).

Begonia erythrophylla 'Beefsteak' is commonly propagated by the conventional methods of vegetative propagation like leaf cuttings. But, to date, there are no reports on the growth

regulator and media requirements, choice of explants and culture conditions for in vitro propagation of 'Beefsteak' begonia.

The objectives of the present study were to regenerate plants from petiole and leaf sections of Begonia erythrophylla 'Beefsteak' using in vitro propagation techniques and suggest a procedure for the mass propagation of the same.

MATERIALS AND METHODS

1. **Stock Plants** : Stock plants of Begonia erythrophylla 'Beefsteak' were maintained in a growth chamber with 16h photoperiod and a constant temperature of 24 ° C. Plants were kept well-watered and fertilized using a liquid solution of 20:20:20.
2. **Explants** : Young, fully expanded leaves (with petioles) were excised from the stock plants and were washed gently with a detergent and running tap water. The surface sterilization procedures were carried out under a laminar flow hood. The washed leaves were then transferred to 20% chlorox (5.25% sodium hypochlorite) solution in 0.1% Tween-20 for 15 minutes. Leaves were then repeatedly washed in sterile water to remove the excess chlorox. Petiole segments (5 mm length) and leaf discs (5 mm diameter) were excised from the sterile leaves and transferred to the culture medium.
3. **Media formulations** : The basal medium consisted of Murashige & Skoog (MS) salts (12) basal medium supplemented with 0.5 mg Nicotinic acid, 0.5 mg Pyridoxine HCl, 0.1 mg Thiamine HCl, 100 mg m-inositol, 30 g sucrose and 10 g agar per liter. The culture medium was supplemented with various combinations of NAA (0, 0.5, 1.0, 2.0 and 5.0 mg/l) and Kinetin (0, 1.0, 2.0, 5.0 and 10.0 mg/l) concentrations. The pH of the medium was adjusted to between 5.6 - 5.7 using 0.1N KOH and autoclaved at 15 psi and

121 °C for 20 minutes. Twenty-five ml aliquots were distributed into 25 x 150 mm sterile test tubes and cooled down to room temperature in an slanting position. A 5 x 5 diallele experiment was employed with 25 treatment combinations and 3 replications.

4. Culture conditions : All culture tubes were placed in an incubator with a constant temperature of 24 °C and light intensity of 1 Klx. Four weeks after initial culture all explants were transferred to a MS shoot multiplication medium (MSSM) consisting of basal MS salts supplemented with 80 mg Adenine sulfate and 30 mg 2iP/l. Observations were recorded at the end of the 8th week on the amount of callus formed and the number of shoots/roots formed (table 2). After 8 weeks of culture the shoots were transferred to a MS rooting medium consisting of basal salts supplemented with 30 g sucrose, 10 g agar and 2 mg IAA/l. Root formation was observed in 2-3 weeks (figure 1c).

5. Transfer of plants to soil : The whole plantlets were transferred to small plastic packs containing 3.75 x 3.75 sq. cm cells (6 cells/pack) filled with sterile vermiculite and placed in large plastic boxes in a growth chamber. The growth chamber was maintained at a constant temperature of 24 °C, 16 h photoperiod and 70 % relative humidity. The boxes were lined with wet paper towels at the bottom and were covered with a ground glass plate to maintain high humidity inside the box. The plantlets were regularly fed with a 10 % Hoagland's nutrient

solution (8) at every watering. The glass plate was gradually lifted up over a period of 2 weeks to expose the plantlets to the growth chamber environment. The hardened plantlets were later transplanted to 10 x 10 sq. cm plastic pots filled with Jiffy-mix and allowed to grow in a greenhouse.

RESULTS & DISCUSSION

Preliminary experiments with NAA (0.0 to 5.0 mg/liter) and Kinetin (0.0 to 10.0 mg/liter) showed that good callus/shoot formation could be obtained with NAA concentrations at 0.5, 1.0 or 2.0 mg/liter and Kinetin concentrations at 2.0, 5.0 or 10.0 mg/liter. No callus/shoot formation was observed in the control medium which lacked growth regulators. Petiole segments responded very well to culture both in terms of callus formation and survival in culture. Leaf discs turned brown and were dead after 2 weeks in culture. So further experiments were carried out using the above concentrations of growth regulators and petiole segments (9 treatment combinations and 3 replications) as explants.

During the first 4 weeks of culture, callus/shoot formation was observed in treatments containing 5.0 mg kinetin + 1.0 or 2.0 mg NAA, 2.0 mg kinetin + 2.0 mg NAA and 10.0 mg kinetin + 1.0 mg NAA (table 1). Green callus was observed in the rest of the treatments. Visual observation showed that the explants (callus) were 3 times the original size at the end of the 4th week in all the treatments except for the lowest (2.0 mg kinetin + 0.5 mg

NAA) and the highest (10.0 mg kinetin + 2.0 mg NAA) Kinetin and NAA concentrations which showed very little or no callus formation. The shoots showed up as rudimentary green protruberances while the callus appeared in bright green clumps. The best callus/shoot formation was observed in the treatments with 5.0 or 10.0 mg kinetin with 1.0 mg NAA (figure 1a).

The explants were transferred after 4 weeks to MS shoot multiplication medium (MSSM) supplemented with 80 mg Adenine sulfate and 30 mg 2iP/liter. The culture conditions were maintained at the same constant temperature of 24°C and 16 h photoperiod. The highest number of shoots (38.67 ± 3.06) per petiole section was obtained in the treatment containing 10.0 mg kinetin + 1.0 mg NAA per liter (figure 1b). Increasing concentrations of kinetin and NAA greatly enhanced shoot formation except for the highest concentration of 10.0 mg kinetin + 2.0 mg NAA which proved to be toxic and killed the callus (table 2). No shoots were obtained from the treatment with 2.0 mg Kinetin and 0.5 mg NAA but the callus still survived. Treatments with 5.0 mg kinetin + all concentrations of NAA and 10.0 mg kinetin + 0.5 mg NAA produced more than 10 shoots per petiole section. The total time lapse between initial culture of the explant and transfer of rooted plantlets to Jiffy-Mix was just under 4 months.

The results conclusively show that successful regeneration of complete plantlets of Begonia erythrophylla 'Beefsteak' can be

obtained through the use of the tissue culture method proposed here. One petiole, divided into 10 explants, can produce about 380 shoots that can be carefully grown into vigorous and well-developed plants within 4-5 months from initial explant culture. Mikkelsen & Sink (10) reported an average of 135 shoots/petiole for Riegior Elatior Begonia 'Schwabenland Red'. Increasing cytokinin/auxin ratios consistently produced higher number of shoots which is in agreement with results obtained by Peck & Cumming (13) for Begonia x tuberhybrida 'Miramichi' and Imelda (7) for Riegior Begonia. Higher concentration (2.0 mg/l) of NAA proved to be toxic to the explants and produced very few shoots in some treatments which is in accordance with results reported by Fannesbech (3) for Begonia x chiemantha. The procedure described in our experiment is faster with higher yields than others reported by Peck and Cumming (13) and Mikkelsen & Sink (10). The results indicate successful in vitro rooting and transfer to soil contrary to those obtained by DeBergh and Maene (2) and Peck and Cumming (13) who reported difficulty in inducing a functioning root system in vitro. A 100 % survival was obtained when plants were transferred to the greenhouse as opposed to 49 % total survival reported by Mikkelsen & Sink (10) for Riegior Elatior Begonia 'Schwabenland Red'.

FIGURE-1 : Response of Begonia erythrophylla 'Beefsteak' to
in vitro culture

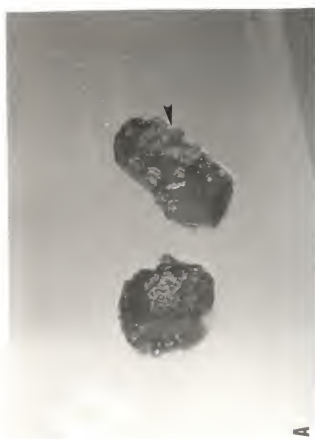
- A : Callus/shoot formation on petiole segments
cultured on MS medium (10.0 mg Kinetin + 1.0 mg
NAA)
- B : Abundant shoot formation on petiole segments
transferred to MS shoot multiplication medium, 8
weeks in culture
- C : Root formation on shoots transferred to MS
rooting medium, 14 weeks in culture
- D : Complete plantlets transferred to sterile
vermiculite medium



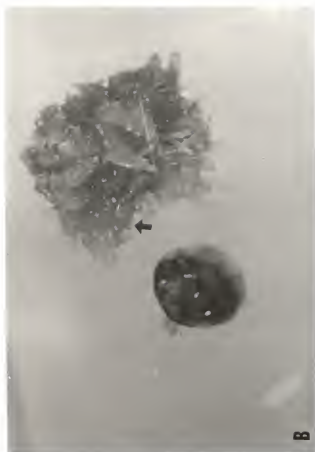
C



D



A



B

Table-1 : Response of petiole segments of B. erythrophylla 'Beefsteak' to various combinations of NAA and Kinetin in MS media after 4 weeks in culture :

Kinetin concn. (mg/liter)	NAA concn. (mg/liter)		
	0.5	1.0	2.0
2.0	callus	callus	callus/shoots
5.0	callus	callus/shoots	callus/shoots
10.0	callus	callus/shoots	callus

Table-2 : Shoot regeneration from petiole segments of B. erythrophylla 'Beefsteak' 4 weeks after transfer to MSSM* medium (8 weeks in culture) :

Kinetin concn. (mg/liter)	Mean shoot number/petiole segment		
	NAA concn. (mg/liter)		
	0.5	1.0	2.0
2.0	0.0	4.0 ± 1.0^1	7.0 ± 1.73
5.0	11.0 ± 1.0	12.67 ± 1.53	10.67 ± 2.08
10.0	12.0 ± 1.73	38.67 ± 3.05	0.0

* = MS shoot multiplication

¹ = mean shoot number \pm standard deviation

LITERATURE CITED

1. Bajaj, Y.P.S. and J.Reinert. 1977. Cryobiology of plant cell cultures and establishment of gene-banks, p.757-776. In: J.Reinert and Y.P.S. Bajaj (eds.). Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-verlag, Berlin.
2. DeBergh, P.C. and L.J. Maene. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. Scientia Hortic. 14 : 335-345.
3. Fonnesbech, M. 1974. Temperature effects on shoot and root development from Begonia x cheimantha petiole segments grown in vitro. Physiol. Plant. 32 : 282-286.
4. Hilding, A. and T. Welander. 1976. Effect of some factors on propagation of Begonia x hiemlis in vitro. Swed. J. Agric. Res. 6 : 191-199.
5. Holdgate, D.P. Propagation of ornamentals by tissue culture. p.18-43. In: J.Reinert and Y.P.S. Bajaj (eds.). Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-verlag, Berlin.
6. Russey, G. 1978. The application of tissue culture to the vegetative propagation of plants. Sci. Prog. 65 : 185-208.
7. Imelda, M. 1983. Tissue culture of Riegor Begonia petiole segments. Annales Bogorienses VIII (1) : 1-11.

8. Johnson, C.M., P.R. Stout, T.C. Broyer and A.B. Carlton. 1957. Comparative chlorine requirements of different plant species. *Plant and Soil* 8 : 337-353.
9. Mellor, F.C., and R. Stace-Smith. 1977. Virus-free potatoes by tissue culture, p.616-636. In: J.Reinert and Y.P.S. Bajaj (eds.). *Applied and fundamental aspects of plant cell, tissue and organ culture*. Springer-verlag, Berlin.
10. Mikkelsen, E.P. and K.C. Sink. 1978. In vitro propagation of Rieger Elatior Begonias. *HortScience* 13 (3) : 242-244.
11. Murashige, T. 1974. Plant propagation through tissue cultures. *Ann. Rev. Plant Physiol.* 25 : 135-166.
12. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth bioassays with tobacco tissue cultures. *Physiol. Plant.* 15 :473-479.
13. Peck, D.E. and B.G. Cumming. 1984. In vitro propagation of Begonia x tuberhybrida from leaf sections. *HortScience* 19(3): 395-397.
14. Skoog, F. and C.O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symp. Soc. Exp. Biol.* 11 : 118-131.
15. Takayama, S. and M. Misawa. 1982. Factors affecting differentiation and growth in vitro and a mass-propagation

- scheme for Begonia x hiemalis. Scientia Hortic. 16 : 65-75.
16. Welander, T. 1981. Effect of polarity on and origin of in vitro formed organs in explants of Begonia elatior hybr. Swed. J. Agric. Res. 11 : 77-83.

APPENDIX I

Media formulations

Media component (mg/liter)	MS ¹	MSSM ²	MSRM ³
<u>Macronutrients</u> :			
NH ₄ NO ₃	1650	1650	1650
KNO ₃	1900	1900	1900
CaCl ₂ ·2H ₂ O	440	332	330
MgSO ₄ ·7H ₂ O	370	181	330
KH ₂ PO ₄	170	170	170
NaH ₂ PO ₄ ·H ₂	--	170	--

Micronutrients :

KI	0.83	0.83	0.83
H ₃ BO ₃	6.20	6.20	6.20
MnSO ₄ ·H ₂ O	22.30	16.90	16.90
ZnSO ₄ ·7H ₂ O	8.60	8.60	8.60
Na ₂ MoO ₄ ·2H ₂ O	0.25	0.25	0.25
CuSO ₄ ·5H ₂ O	0.025	0.016	0.016
CoCl ₂ ·6H ₂ O	0.025	0.025	0.025
FeSO ₄ ·7H ₂ O (EDTA)	43.0	36.70	36.70
Adenine·SO ₄ ·2H ₂ O	--	80.00	--

Vitamins & Hormones :

M-inositol	100	100	100
Nicotinic acid	0.5	--	--
Pyridoxine.HCl	0.5	--	--
Thiamine.HCl	0.1	0.4	0.4
IAA	--	0.3	2.0
NAA	0.0 - 5.0	--	--
Kinetin	0.0 - 10.0	--	--
2iP	--	30.0	--

....contd.

- ¹ = Murashige & Skoog Medium
² = MS shoot multiplication medium
³ = MS rooting medium

APPENDIX I (contd.)

Media component (mg/liter)	MS	MSSM	MSRM
<u>Sugar :</u>			
Sucrose	30,000	30,000	30,000
<u>Others :</u>			
Glycine	2.0	--	--
Agar	10,000	8,000	8,000
pH	5.6 - 5.7	5.6 - 5.7	5.6 - 5.7

APPENDIX II

Experimental Design

A 5 x 5 diallele experiment was adopted for both Peperomia and Begonia species with 25 treatment combinations and 3 replications. The treatment (T₁) with no added growth regulators served as the control. Statistical analysis was limited to the calculation of mean and standard deviations.

Kinetin concn. (mg/liter)	NAA concn. (mg/liter)				
	0.0	0.5	1.0	2.0	5.0
0.0	T ₁	T ₂	T ₃	T ₄	T ₅
1.0	T ₆	T ₇	T ₈	T ₉	T ₁₀
2.0	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₁₅
5.0	T ₁₆	T ₁₇	T ₁₈	T ₁₉	T ₂₀
10.0	T ₂₁	T ₂₂	T ₂₃	T ₂₄	T ₂₅

APPENDIX III a

Results of experiments repeated

Response of petiole segments of *P. argyreia* 'Watermelon' four weeks after transfer to MSSM* medium (8 weeks in culture) :

Kinetin concn. (mg/l)	NAA concn. (mg/l)				
	0.0	0.5	1.0	2.0	5.0
0.0	-- ¹	--	--	callus	--
1.0	--	--	c/r ²	c/r	--
2.0	--	--	NR ³	c/r	--
5.0	--	NR	c/r	roots	--
10.0	--	--	NR	shoots (12.33±0.58) ⁴	--

* = Murashige & Skoog shoot multiplication

1 = dead explant

2 = callus/roots

3 = no response

4 = mean shoot number ± standard deviation

APPENDIX III b

Results of experiments repeated

Shoot regeneration from petiole segments of P. caperata
'Emerald Ripple' four weeks after transfer to MSSM* medium
(8weeks in culture):

Kinetin concn. (mg/l)	Mean shoot number/petiole segment				
	NAA concn. (mg/l)				
	0.0	0.5	1.0	2.0	5.0
0.0	-- ¹	--	--	--	--
1.0	--	--	--	--	--
2.0	--	--	1.67±0.58 ²	2.33±1.53	--
5.0	2.0±1.0	2.33±1.15	5.33±0.57	7.66±0.58	--
10.0	2.33±1.15	4.66±0.57	9.67±2.08	3.33±0.58	--

* = Murashige & Skoog shoot multiplication

1 = dead explant

2 = mean shoot number ± standard deviation

ACKNOWLEDGEMENTS

I am deeply indebted to my major professor, Dr. H. Khataian, for his invaluable advice, constant guidance, patience and enthusiasm during my course of study. My sincere thanks and appreciation to him.

My special thanks go to Dr. C.B. Rajashekar, Department of Horticulture and Dr. J.A. Guikema, Division of Biology, for their comments, advice and support as members of my supervisory committee. I wish to thank Dr. L.B. Johnson, Department of Plant Pathology, for his help and advice in carrying out my research.

I also take this opportunity to thank the Department of Horticulture and the Division of Biology for providing me with financial support to carry out my graduate studies.

Lastly, I am greatly indebted to my parents and brother in India for their moral support, love and encouragement during my stay here in the United States. I dedicate this thesis to my parents.

IN VITRO PROPAGATION OF PEPEROMIA AND BEGONIA SPECIES

by

SRINIVASA RAMACHANDRA

B.Sc.(Agri.), University of Agricultural Sciences

Bangalore, India, 1982

AN ABSTRACT OF A MASTER'S THESIS
submitted in partial fulfillment of
the requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

ABSTRACT

Leaf discs and petiole sections of Peperomia argyreia 'Watermelon', P. caperata 'Emerald Ripple' and Begonia erythrophyllum 'Beefsteak' were in vitro cultured on MS medium supplemented with different levels of NAA and Kinetin. Explants were transferred to MS shoot multiplication medium 4 weeks after initial culture. Four weeks later the regenerated shoots were transferred to MS rooting medium. Whole plantlets were transferred to 7.5 x 7.5 cm plastic packs filled with sterile vermiculite. The plantlets were placed in plastic boxes lined with wet paper towels and covered with ground glass plate and placed in a growth chamber at 16 h photoperiod and temperature of 24 ° C.

Whole plantlets were regenerated from leaf discs of 'Emerald Ripple' when cultured on a MS medium supplemented with different levels of NAA and kinetin. 'Watermelon' leaf discs showed poor response to in vitro culture except for the highest concentration of NAA and Kinetin which gave whole plantlets. 'Beefsteak' Begonia regenerated about 40 plantlets per petiole section. A 100 % survival was obtained when whole plantlets of Begonia were transferred to vermiculite medium.